

*Renumbered
cems
2/12/04*

In the claims:

This listing of claims will replace all prior versions, and listings, of claims in the application.

1. (currently amended) A method for producing ~~a vaccine~~ an antigen delivery system comprising a plurality of polymer particles, wherein a water-insoluble protein antigen is incorporated with the polymer particles, the polymer particles comprising a matrix polymer which comprises one or more homo- and/or copolymers, wherein the method comprises:

(a) mixing an aqueous phase (W) comprising the water-insoluble protein and one or more ~~solubilizing agents~~ hydrophilic surfactants at a concentration of 0.1 to 100 times the critical micelle concentration thereof with an organic phase (O) that comprises the matrix polymer in an organic solvent, which solvent does not denature the protein antigen and wherein O is immiscible with W, to produce a W/O emulsion, wherein either W or O or both further comprise one or more stabilizing agents added prior to mixing to stabilize the W/O emulsion in the presence of the solubilizing agent(s) and promote the incorporation of the water-insoluble protein within the polymer particles during step (b); and

(b) forming droplets of said W/O emulsion by dispersing the emulsion in a fluid medium, and removing said solvent from the O phase of the W/O emulsion droplets to thereby form the polymer particles incorporating the water-insoluble protein antigen.

2. (previously presented) The method of claim 1, wherein more than one stabilizing agent is included in the W/O emulsion.

~~5~~ 3. (previously presented) The method of claim 1 or 2, wherein the one or more stabilizing agents is/are selected from the group consisting of polymers, polar lipids, and hydrophobic surfactants.

~~6~~ 4. (currently amended) The method of claim ~~3~~⁵, wherein the one or more stabilizing agents is/are a polymer selected from the group consisting of poly(vinyl pyrrolidone), poly(vinyl alcohol), polysaccharides, polyethyleneoxide and water-soluble proteins.

~~7~~ 5. (previously presented) The method of claim ~~3~~⁵, wherein the one or more stabilizing agents is/are a polar lipid selected from the group consisting of cholesterol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, glycolipids and phosphatidic acid.

~~8~~ 6. (previously presented) The method of claim ~~3~~⁵, wherein the one or more stabilizing agents is/are a non-ionic, hydrophobic

surfactant selected from the group consisting of a sorbitan fatty acid ester, hydrophobic polyoxyethylene alkyl ether, sucrose ester, alkyl-glucopyranoside, polyglycerol polyricinoleate and block-copolymers of ethylene oxide with propyleneoxide and/or lactic acid.

9. (previously presented) The method of claim ~~8~~⁵, wherein the one or more stabilizing agents is/are an anionic, hydrophobic surfactant selected from the group consisting of an alkylsulphate salt, a dialkylsulphosuccinate salt, an alkylbenzene sulphonate salt and a fatty acid salt.

10. (previously presented) The method of claim ~~8~~⁵, wherein the one or more stabilizing agents is/are a cationic, hydrophobic surfactant selected from the group consisting of an alkyltrimethylammonium salt and a dialkyldimethylammonium salt.

3. (previously presented) The method of claim 2, wherein one of the stabilizing agents is a sorbitan fatty acid ester.

4. (previously presented) The method of claim 2, wherein the stabilizing agents comprise poly (vinyl pyrrolidone) and sodium 1,4-bis(2-ethylhexyl) sulphosuccinate.

11. (previously presented) The method of claim 1, wherein the aqueous phase comprises more than one solubilizing agent.

12. (canceled)

12/

13. (currently amended) The method of claim 12 1, wherein the hydrophilic surfactant is a non-ionic surfactant selected from the group consisting of alkyl-glucopyranosides, alkyl-thioglucofuranosides, alkyl-maltosides, alkyl-methyl glucamides, glucamides, polyoxyethylene alcohols, polyoxyethylene alkyl phenols, emulphogens, polyoxyethylene sorbitol esters, polyoxyethylene fatty acid esters, hydrophilic polyoxyethylene alkyl ethers and digitonin.

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14. (currently amended) The method of claim 12 1, wherein the hydrophilic surfactant is an anionic surfactant selected from the group consisting of cholates, alkylsulphonates, deoxycholates, alkylsulphates, oligoxyethylene dodecyl ether sulphates and sodium dodecylsarcosinate.

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15. (currently amended) The method of claim 12 1, wherein the hydrophilic surfactant is a cationic surfactant selected from the group consisting of alkylpyridinium salts and alkyltrimethylammonium salts.

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16. (currently amended) The method of claim 12 1, wherein the hydrophilic surfactant is a zwitterionic surfactant selected from the group consisting of 3-1-propanesulphonate (CHAPS), 3-[(3-cholamidopropyl)-dimethylammonio]-2-hydroxy-1-propanesulphonate (CHAPSO), N,N-bis-cholamide (BIGCHAP), N,N-bis-deoxycholamide (deoxy BIGCHAP), lyso phosphatidylcholine, alkylbetaines and sulphobetaines.

17. (canceled)

18. (canceled)

~~16~~ 19. (previously presented) The method of claim 1 which includes a Double Emulsion (W/O/X) Solvent Evaporation Technique wherein the fluid medium in which the stabilized W/O emulsion is dispersed in step (b) is a liquid phase (X) which is immiscible with the O phase, said method producing a W/O/X double emulsion comprising W/O droplets from which the solvent is evaporated.

~~17~~ 20. (previously presented) The method of claim 1 which includes a Double Emulsion (W/O/X) Solvent Extraction Technique wherein the fluid medium in which the stabilized W/O emulsion is dispersed in step (b) is a liquid phase (X) which is immiscible with the O phase, said method producing a W/O/X double emulsion comprising W/O droplets, and wherein the removal of the organic solvent from the O phase of the droplets is achieved through extraction by the X phase.

~~18~~ 21. (previously presented) The method of claim ~~15~~ ¹⁶ or ~~20~~ ¹⁷, wherein the X phase comprises a stabilizing agent.

~~19~~ 22. (previously presented) The method of claim ~~21~~ ¹⁸, wherein the one or more stabilizing agents is/are selected from group consisting of polymers, polar lipids, and hydrophobic surfactants.

~~25~~
23. (previously presented) The method of claim 1, wherein the dispersal of the stabilized W/O emulsion in a fluid medium during polymer formulation in step (b) is achieved with a spray drying technique, wherein the stabilized W/O emulsion is dispersed in a gaseous medium to form a spray of W/O emulsion droplets from which said solvent evaporates.

~~26~~
24. (previously presented) The method of claim 1, wherein the dispersal of the stabilized W/O emulsion in a fluid medium during polymer particle formulation in step (b) is achieved with a fluid gas technique.

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25. (previously presented) The method of claim ~~24~~²⁶, wherein the fluid gas technique is selected from the group consisting of gas anti-solvent precipitation (GAS), solution enhanced dispersion by supercritical fluid (SEDS), precipitation with compressed anti-solvents (PCA), supercritical anti-solvent (SAS) and aerosol solvent extraction system (ASES).

~~28~~
26. (previously presented) The method of claim 1, wherein the protein antigen is a *Helicobacter* protein or *Helicobacter* protein fragment.

~~29~~
27. (previously presented) The method of claim ~~26~~²⁸, wherein the *Helicobacter* protein or *Helicobacter* protein fragment is from *Helicobacter pylori*.

~~30~~ 28. (original) The method of claim ~~28~~ ^{28 29} or ~~27~~, wherein said *Helicobacter* protein is a protein expressed on the surface of *Helicobacter*.

~~32~~ 29. (previously presented) The method of claim ~~28~~ ³⁰, wherein the *Helicobacter* protein is a lipidated form of *Helicobacter pylori* adhesion antigen (HpaA).

~~33~~ 30. (original) The method of claim ~~29~~, wherein the protein is a fully lipidated form of HpaA.

~~31~~ 31. (original) The method of claim ~~29~~ ³⁰, wherein the protein part of the lipidated HpaA protein has an amino acid sequence that is identical to, or substantially similar to, positions 28 to 260 of SEQ ID NO. 2 or 4.

~~34~~ 32. (currently amended) The method of claim 1, wherein the matrix polymer is a ~~homo- or co-polymer~~ selected from one or more of the group consisting of polyesters, polyanhydrides, polyorthoesters, polycarbonates, polyamides, poly(amino acids), polyacetals, polycyanoacrylates, polyacrylates, biodegradable polyurethanes, non-erodible polyurethanes, polymers of ethylene-vinyl acetate, acyl substituted cellulose acetates, polysaccharides, polystyrenes, polyvinyl chloride, polyvinyl fluoride, poly(vinyl imidazole), chlorosulphonated polyolefins, polyethylene oxide, polyethers and polyoxalates.

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33. (currently amended) The method of claim 32, wherein the matrix polymer is a polyester homopolymer selected from the group consisting of polylactic acid, polyglycolic acid, polyhydroxybutyrate, poly(alpha hydroxyacids) and polycaprolactone.

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34. (currently amended) The method of claim 32, wherein the matrix polymer is a polyester co-polymer selected from the group consisting of poly(lactide-co-glycolide), poly(lactic-co-glycolic acid), poly(hydroxybutyrate-hydroxyvalerate) and poly(lactide-co-caprolactone).

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35. (original) The method of claim 34, wherein the matrix polymer is poly(D,L-lactide-co-glycolide).

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36. (previously presented) The method of claim 1, wherein in step (a) the W phase is mixed with the O phase in a ratio by volume of 1:100 to 1:1.

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37. (currently amended) An antigen vaccine delivery system produced by the method of claim 1, wherein the one or more stabilizing agents is/are a polymer selected from the group consisting of poly(vinyl pyrrolidone), poly(vinyl alcohol), polysaccharides, polyethyleneoxide and water soluble proteins, and wherein the method includes a Double Emulsion (W/O/X) Solvent Evaporation Technique wherein the fluid medium in which the stabilized W/O emulsion is dispersed in step (b) is a liquid phase (X) which is immiscible with the O phase, said

method producing a W/O/X double emulsion comprising W/O droplets from which the solvent is evaporated.

38-44. (canceled)

41/45. (currently amended) The vaccine delivery system of claim 37, wherein the matrix polymer is a ~~homo- or co-polymer~~ selected from one or more of the group consisting of polyesters, polyanhydrides, polyorthoesters, polycarbonates, polyamides, poly(amino acids), polyacetals, polycyanoacrylates, polyacrylates, biodegradable polyurethanes, non-erodible polyurethanes, polymers of ethylene-vinyl acetate, acyl substituted cellulose acetates, polysaccharides, polystyrenes, polyvinyl chloride, polyvinyl fluoride, poly(vinyl imidazole), chlorosulphonated polyolefins, polyethylene oxide, polyethers and polyoxalates.

42/46. (currently amended) The vaccine antigen delivery system of claim 45, wherein the polymer is a polyester homopolymer selected from the group consisting of polylactic acid, polyglycolic acid, polyhydroxybutyrate, poly(alpha hydroxyacids) and polycaprolactone.

43/47. (currently amended) The vaccine antigen delivery system of claim 45, wherein the matrix polymer is a polyester co-polymer selected from the group consisting of poly(lactide-co-glycolide), poly(lactic-co-glycolic acid),

poly(hydroxybutyrate-hydroxyvalerate) and poly(lactide-co-caprolactone).

~~44~~ 48. (currently amended) The vaccine antigen delivery system of claim ~~47~~⁴³, wherein the matrix polymer is poly(D,L-lactide-co-glycolide).

~~45~~ 49. (currently amended) The vaccine antigen delivery system of any one of claims ~~37 and 45-48~~^{40, 41, 42, 43, or 44}, wherein the polymer particles have an average diameter of 0.05-20 μ m according to the volume size distribution.

~~53~~ 50. (currently amended) A An immunogenic composition comprising the vaccine delivery system of any one of claims ~~37~~⁴⁰, and ~~45-48~~ 41, 42, 43, or 44.

~~57~~ 51. (currently amended) A method for ~~the treatment of~~ inducing an immune response directed against existing *Helicobacter* infection in a mammalian host, comprising administering to the mammalian host an effective amount of the composition according to claim ~~50~~⁵³ wherein the water-insoluble protein antigen is a *Helicobacter* antigen.

~~54~~ 52. (currently amended) A method for inducing an immune response directed toward preventing or reducing the risk of *Helicobacter* infection in a mammalian host, comprising administering to the mammalian host an effective amount of the

~~53~~

composition according to claim ~~50~~ wherein the water-insoluble protein antigen is a *Helicobacter* antigen.

~~20~~ ~~53~~. (previously presented) The method of claim ~~21~~¹⁸, wherein the one or more stabilizing agents is/are a polymer selected from the group consisting of poly(vinyl pyrrolidone), poly(vinyl alcohol), polysaccharides, polyethyleneoxide and water soluble proteins.

~~21~~ ~~54~~. (previously presented) The method of claim ~~21~~¹⁸, wherein the one or more stabilizing agents is/are a polar lipid selected from the group consisting of cholesterol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, glycolipids and phosphatidic acid.

~~22~~ ~~55~~. (previously presented) The method of claim ~~21~~¹⁸, wherein the one or more stabilizing agents is/are a non-ionic, hydrophobic surfactant selected from the group consisting of sorbitan fatty acid ester, hydrophobic polyoxyethylene alkyl ether, sucrose ester, alkyl-glucopyranoside, polyglycerol polyricinoleate and block-copolymers of ethylene oxide with propyleneoxide and/or lactic acid.

~~23~~ ~~56~~. (previously presented) The method of claim ~~21~~¹⁸, wherein the one or more stabilizing agents is/are an anionic, hydrophobic surfactant selected from an alkylsulphate salt, dialkylsulphosuccinate salt, alkylbenzene sulphonate salt and a fatty acid salt.

²⁴ 57. (previously presented) The method of claim ¹⁸ 21, wherein the one or more stabilizing agents is/are a cationic, hydrophobic surfactant selected from the group consisting of an alkyltrimethylammonium salt and a dialkyldimethylammonium salt.

⁴⁶ 58. (currently amended) A An immunogenic composition comprising the ~~vaccine~~ delivery system of claim ⁴⁵ 45.

⁵⁰ 59. (currently amended) A method for ~~the treatment of~~ inducing an immune response directed against existing *Helicobacter* infection in a mammalian host comprising administering to the mammalian host an effective amount of the composition according to claim ⁴⁶ 58 wherein the water-insoluble protein antigen is a *Helicobacter* antigen.

⁴⁷ 60. (currently amended) A method for inducing an immune response directed toward preventing or reducing the risk of *Helicobacter* infection in a mammalian host, comprising administering to the mammalian host an effective amount of the composition according to claim ⁴⁶ 59 wherein the water-insoluble protein antigen is a *Helicobacter* antigen.

⁶⁰ 61. (new) The composition according to claim ⁵³ 50 wherein the protein antigen is a *Helicobacter* antigen.

~~60~~ 62. (new) The composition according to claim ~~61~~ ⁶⁰ wherein the protein antigen is a lipidated form of *Helicobacter pylori* adhesion antigen (HpaA).

~~62~~ 63. (new) The composition according to claim ~~62~~ ⁶¹ wherein the protein part of the lipidated antigen has an amino acid sequence that is identical to, or substantially similar to, positions 28 to 260 of SEQ ID NO. 2 or 4.

~~63~~ 64. (new) The composition according to claim ~~58~~ ⁴⁶ wherein the protein antigen is a *Helicobacter* antigen.

~~64~~ 65. (new) The composition according to claim ~~64~~ ⁶³ wherein the protein antigen is a lipidated form of *Helicobacter pylori* adhesion antigen (HpaA).

~~65~~ 66. (new) The composition according to claim ~~65~~ ⁶⁴ wherein the protein part of the lipidated antigen has an amino acid sequence that is identical to, or substantially similar to, positions 28 to 260 of SEQ ID NO. 2 or 4.

~~58~~ 57. (new) The method according to claim ~~51~~ ⁵⁷ wherein the protein antigen is a lipidated form of *Helicobacter pylori* adhesion antigen (HpaA).

~~59~~ 58. (new) The method according to claim ~~57~~ ⁵⁸ wherein the protein part of the lipidated antigen has an amino acid sequence that is identical to, or substantially similar to, positions 28 to 260 of SEQ ID NO. 2 or 4.

~~55~~ 59. (new) The method according to claim ~~52~~ ⁵⁴ wherein the protein antigen is a lipidated form of *Helicobacter pylori* adhesion antigen (HpaA).

~~56~~ 60. (new) The method according to claim ~~53~~ ⁵⁵ wherein the protein part of the lipidated antigen has an amino acid sequence that is identical to, or substantially similar to, positions 28 to 260 of SEQ ID NO. 2 or 4.

~~51~~ 61. (new) The method according to claim ~~50~~ ⁵⁰ wherein the protein antigen is a lipidated form of *Helicobacter pylori* adhesion antigen (HpaA).

~~52~~ 62. (new) The method according to claim ~~51~~ ⁵¹ wherein the protein part of the lipidated antigen has an amino acid sequence that is identical to, or substantially similar to, positions 28 to 260 of SEQ ID NO. 2 or 4.

~~48~~ 63. (new) The method according to claim ~~50~~ ⁴⁷ wherein the protein antigen is a lipidated form of *Helicobacter pylori* adhesion antigen (HpaA).

~~49~~ 64. (new) The method according to claim ~~53~~ ⁴⁸ wherein the protein part of the lipidated antigen has an amino acid sequence that is identical to, or substantially similar to, positions 28 to 260 of SEQ ID NO. 2 or 4.

~~38~~ 65. (new) The method according to claim 1 wherein the organic solvent in the organic phase (O) is selected from the group

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consisting of methylene chloride, chloroform and ethyl
acetate.